Coupled Solute Fluxes in Toad Skin

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ABSTRACT Net inward flux of mannitol across toad skin induced by making the outside solution hypertonic with urea has been investigated. No significant relation between net mannitol flux and net Na flux could be detected when both fluxes were measured simultaneously. In addition, the net mannitol flux caused by hypertonic solution was not altered by inhibition of Na transport with ouabain or by replacement of all Na in the bathing solutions by choline. The rate of net mannitol flux was dependent on the magnitude of the urea concentration difference across the skin and the direction of net flux could be reversed by reversing the direction of the urea concentration difference. These observations suggest that the mannitol transfer is the result of a coupling between the flows of urea and mannitol.

Ussing (1) has recently reported an unexpected effect on solute transfer across the isolated frog skin when the outside bathing solution is made hypertonic with urea or other solutes. Under these conditions, net inward flow of sucrose was observed when both bathing solutions contained equal concentrations of sucrose. Similar results were obtained for sulfate in short-circuited skins. Ussing also observed a correlation between the magnitude of this net sucrose transfer and the short-circuit current and suggested that the "apparent active transfer" of sucrose might be related to active Na transport. This conclusion seemed to be supported by observations that net transfer of sucrose was abolished by cyanide and by replacement of Na in the bathing solutions by K. Franz and Van Bruggen (2) have reported a similar effect on several organic solutes but have questioned the role of Na transport in the phenomenon. They suggested that the net solute transfer could be caused by a drag arising from the diffusion of the hypertonic agent itself from a high concentration in the outside solution to a low one in the inside solution.

The present experiments were carried out to examine these possibilities in more detail. When the solution bathing the outside of the frog skin is made hyperosmotic with urea, there is a discrepancy between short-circuit current and net Na transport (1). Consequently, in order to examine the relation between net Na transport and the induced solute flux under these conditions,

net fluxes of Na and mannitol were determined simultaneously using four tracers. The rate of Na transport was changed by varying Na concentration in the bathing media or by adding ouabain. In addition, relations between urea concentration in the outside solution and mannitol fluxes and between urea fluxes and mannitol fluxes were examined.

METHODS

The abdominal skin of *Bufo marinus* was mounted in a chamber described by Kidder et al. (3) designed to reduce solution volume to 5 ml on each side and facilitate the measurement of small unidirectional fluxes. The area of skin exposed to bathing solution was 3.14 cm^2. Toad skin was used in these studies because in preliminary experiments hypertonic outside solutions caused a greater asymmetry in unidirectional mannitol fluxes in this tissue than in frog skin. Electrical potential difference across the skin and short-circuit current were measured as previously described (4). The normal Ringer solution used contained 112 mM NaCl, 2.5 mM $KHCO_3$, 1.0 mM $CaCl_2$, and in most experiments, 1 mM mannitol and had a pH of 8.1 when equilibrated with air. In experiments in which Na concentration was reduced, NaCl was replaced by an equivalent concentration of choline chloride. Hypertonic solutions were prepared by adding urea to the appropriate Ringer solution to give urea concentrations of 100–400 mM.

Na influx, Na outflux, mannitol influx, and mannitol outflux were measured simultaneously by adding ^{24}Na (5–10 μc) and D-mannitol-1-^{3}H (30 μc) to the outside bathing solution and ^{22}Na (0.25 μc) and D-mannitol-1-^{14}C (1 μc) to the inside bathing solution. Every hour, 1 ml samples were collected from the inside solution, dried on planchets, and counted in a gas flow counter to determine ^{24}Na influx. The ^{24}Na activity in the samples was determined with the technique described by Biber et al. (5). Another 1 ml sample was collected from each side at hourly intervals, and diluted in 15 ml of Bray's solution (6). Ringer's solution containing the original specific activity was used to refill the chambers to the initial volume. "Hot side" samples were taken hourly or at the beginning and end of the experiment. They were diluted with nonradioactive fluid having the chemical composition of the solution bathing the opposite side of the skin in order to minimize differences in quenching between "hot" and "cold" side samples. After allowing 14 days for decay of ^{24}Na, all samples were counted in a three channel liquid scintillation counter to determine activities of ^{3}H, ^{14}C, and ^{22}Na. Although the ratios of the activities of the three isotopes in the samples were not ideal for separation, adequate settings of the counter could be achieved and reproducible results were usually obtained. Standards containing single isotopes in the appropriate solutions were counted with each experiment.

In some experiments, only two isotopes were used. Na influx and outflux were measured simultaneously by adding ^{22}Na (0.25 μc) to the outside and ^{24}Na (15–25 μc) to the inside solutions. Both solutions were sampled every 30 min. The samples were dried on planchets and counted in a gas flow counter as previously described (5). In another group of experiments, mannitol influx and outflux were measured in the same skin by adding D-mannitol-1-^{3}H (30 μc) to the outside solution and D-mannitol-1-^{14}C (1 μc) to the inside. Samples were taken at 40 min intervals, placed in Bray's

solution, and counted in a liquid scintillation counter. Urea and mannitol influxes were determined simultaneously in a separate set of experiments in which urea-^{14}C (0.5 μc) and mannitol-^{3}H (30 μc) were added to the outside solution. In all cases, fluxes were calculated from the rate of tracer appearance on the cold side and the specific activity of that tracer on the hot side.

RESULTS

Comparison of Mannitol-^{3}H and Mannitol-^{14}C Fluxes

In order to test whether flux determinations with the two mannitol tracers gave the same results, mannitol-^{3}H and mannitol-^{14}C were added simultaneously to the outside solution in one experiment and to the inside solution in another. The skins were bathed with Na-free choline medium on both sides; the inside solution also contained urea and mannitol at concentrations of 1 mM while the outside solution contained 1 mM mannitol and 200 mM urea. The results given in Table I indicate that the unidirectional mannitol fluxes calculated from ^{3}H and ^{14}C flows did not differ appreciably. Thus, any net flux of mannitol observed in experiments in which influx and outflux are measured with different tracers cannot be ascribed to anomalous behavior of the tracers.

TABLE I

FLUXES MEASURED BY ^{3}H- AND MANNITOL-^{14}C*

	Mannitol influx		Mannitol outflux	
Time	^{14}C	^{3}H	^{14}C	^{3}H
hr	*μmole/hr cm^2*		*μmole/hr cm^2*	
1	0.041	0.040	0.0037	0.0039
2	0.015	0.015	0.0062	0.0061
3	0.013	0.012	0.0073	0.0070

*Fluxes of both tracers were measured simultaneously in the same skin.

Correlation between Na Flux and Mannitol Flux

In two experiments, the rate of net sodium movement was varied by exposing both sides of the skin for three 1 hr periods to 15 mM Na and then for three 1 hr periods to 112 mM Na. In three experiments, the protocol was reversed so that the skins were exposed first to 112 mM Na and then to 15 mM Na. In all cases, the outside solution contained 200 mM urea, and both solutions contained 1 mM mannitol. Net fluxes of Na and mannitol were determined simultaneously using four isotopes to measure the unidirectional fluxes. In each flux period, mannitol influx exceeded outflux and a net flux ranging from 0.003 to 0.019 μmole/hr cm^2 was observed in agreement with the findings

of Franz and Van Bruggen (2). The relation between the net fluxes of Na and mannitol for the 30 observation periods in these experiments is shown in Fig. 1. The linear regression line calculated from these points is

$$J_m = [(3.6 \pm 6.1) \times 10^{-4}]J_{Na} + 0.0098 \pm 0.0010$$

where J_m is net mannitol flux and J_{Na} is net Na flux. Standard errors for the slope and intercept are included. The slope of this line is not significantly different from zero and the correlation coefficient is 0.11 suggesting that there

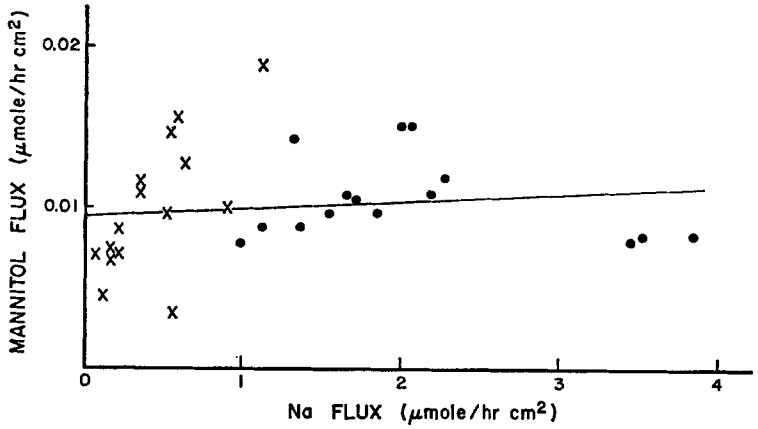

FIGURE 1. Relation between net mannitol flux and net Na flux. Each point represents a single flux period in which both net fluxes were measured simultaneously at 115 mM (●) or 15 mM (×) Na. In all experiments, the outside solution contained 200 mM urea and both solutions contained 1 mM mannitol. The line was determined by least squares.

is no correlation between the two fluxes. Thus, we are unable to demonstrate a relation between the mannitol flux induced by a hypertonic outside solution and the simultaneously measured Na flux. There is, however, an appreciable scatter in the data obtained in these experiments, due in part to the difficulties inherent in the multiple tracer technique, and a small slope might escape detection. We have, therefore, used other approaches in an effort to examine this possible relation further.

Effect of Ouabain

The effects of ouabain on Na and mannitol fluxes are summarized in Table II. The upper half of the table shows the results of experiments with the skin bathed on both sides with normal Na Ringer's. Fluxes were measured for three control periods, ouabain was added to the inside solution, and flux measurements were continued. The first 30 min period after addition of ouabain has not been included in the average. Under control conditions, the

short-circuit current does not differ significantly from net Na flux. Addition of ouabain (10^{-3} M) caused a marked decrease in both net Na flux and short-circuit current.

The lower half of Table II shows the results of similar experiments in which the outside solution contained 200 mM urea. Unidirectional Na and mannitol fluxes were measured simultaneously and ouabain was added after three control periods. In the presence of 200 mM urea, net Na flux was appreciably lower than the value observed when the skins were bathed in normal Ringer's. This difference appeared to be due primarily to an eightfold increase in Na outflux under hypertonic conditions. In addition, the net Na flux was significantly greater than the short-circuit current, in agreement with the obser-

TABLE II
EFFECTS OF OUABAIN

	Na fluxes				Mannitol fluxes		
	Influx	Outflux	Net	I*	Influx	Outflux	Net
	μeq/hr cm²			*μeq/hr cm²*	*μmole/hr cm²*		
Control (11)	5.03	0.32	4.71±0.26‡	4.83±0.18	—	—	—
+ Ouabain (6)	1.58	1.03	0.55±0.35	0.55±0.08	—	—	—
+ Urea (12)	3.98	2.50	1.48±0.24	0.49±0.07	0.025	0.007	0.018±0.002
+ Urea + ouabain (12)	3.67	3.28	0.39±0.08	0.15±0.02	0.023	0.010	0.013±0.002

* Short-circuit current.

‡ Errors are given as standard error of the mean. Number of observations given in parentheses.

vations of Ussing on frog skin (1). Addition of ouabain caused a further decline in net Na flux and current, but did not cause a significant change in net mannitol flux ($0.05 < p < 0.1$). The observed decrease in net mannitol flux may be due in part to the fact that measurement in the presence of ouabain must necessarily follow the control measurements. As indicated by the average unidirectional fluxes shown in Fig. 2, there was usually a decline in net mannitol flux over the first 3 hr of exposure of the skin to urea due to a progressive rise in outflux. The average value of net mannitol flux in the period just prior to addition of ouabain was 0.014 μmole/hr cm² which is close to the value of 0.013 observed after ouabain treatment. Thus inhibition of Na transport with ouabain does not lead to a decrease in the mannitol flux induced by urea.

Mannitol Flux in the Absence of Na

The regression line relating Na and mannitol fluxes suggests that there would be a significant net mannitol flux in the absence of Na in the bathing solutions. However, inspection of Fig. 1 also discloses the possibility that there could

be a sharp decline in mannitol flux at low Na fluxes. Consequently, experiments were carried out to determine net mannitol flux in skins bathed on both sides with Na-free choline Ringer's. As in experiments using Na-containing solutions, the outside solution contained 200 mM urea. The results of these experiments, together with those at 15 and 112 mM Na, are summarized in Table III. Urea in the outside solution induced a net inward mannitol flux in the absence of Na that did not differ from the flux observed at 112 mM Na. In addition, the net flux observed in Na-free solution agrees well with the value expected from the intercept of the line in Fig. 1.

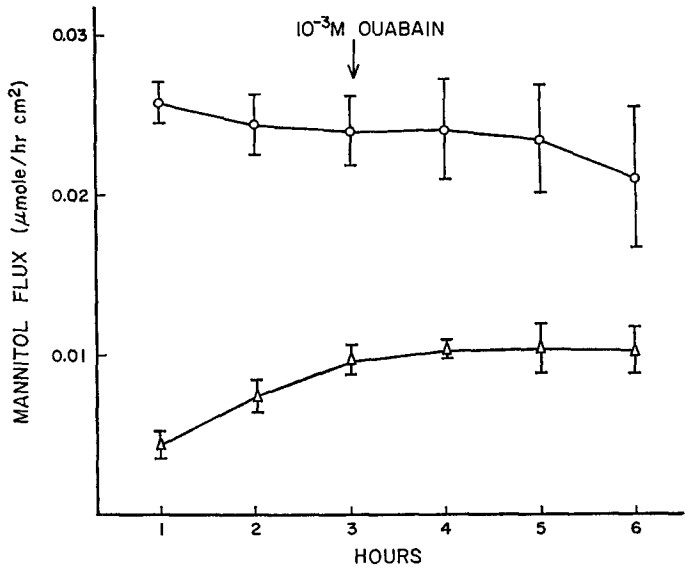

Figure 2. Unidirectional mannitol fluxes (○, influx; △, outflux) as a function of time. Each point is the average of 12 determinations and bars represent ±1 SE.

Effect of Urea Concentration on Mannitol Fluxes

Seven experiments were carried out to examine the effect on unidirectional mannitol fluxes of changes in the urea concentration of the outside solution. Skins were bathed on both sides with choline Ringer's and three urea concentrations were tested on each skin. Fluxes were measured for a single 1 hr period at each concentration and an equilibration period of 30–40 min was allowed following change of urea concentration. Results are summarized in Fig. 3. Both fluxes increase nearly linearly with urea concentration but the slope is much steeper for influx. Since in all experiments urea concentrations were tested in the order 100, 200, 400 mM, the observed increase in outflux may be due in part to time of exposure of the skin to hypertonic solutions.

TABLE III

EFFECT OF Na CONCENTRATION ON MANNITOL FLUX

[Na]	Net mannitol flux	n*
mM	μmole/hr cm²	
112	0.012±0.001‡	46
15	0.010±0.001	15
Na-free	0.011±0.002	16

* Number of observations.

‡ Standard error of the mean.

As shown in Fig. 2, increases in outflux of similar magnitude were observed over a 3 hr period of exposure to 200 mM urea. Further, increased urea concentrations should cause an increase in water flow from inside to outside and any solvent drag effect on mannitol would tend to increase outflux and decrease influx. Thus, the direct effect of urea on mannitol efflux may be overestimated and the effect on influx underestimated. Lines drawn through the points intersect the y axis at approximately the same point suggesting that there will be no net flux in the absence of the hypertonic agent.

Fig. 4 shows the relation between influxes of urea and mannitol measured simultaneously in skins bathed in choline Ringer's. The urea concentration in the outside solution was changed successively from 1 to 100 to 200 and 400 mM but the mannitol concentration was always 1 mM. Fluxes were measured for two 30 min periods at each urea concentration and a 30 min equilibration period followed each concentration change. Mannitol influx is approximately a linear function of urea influx.

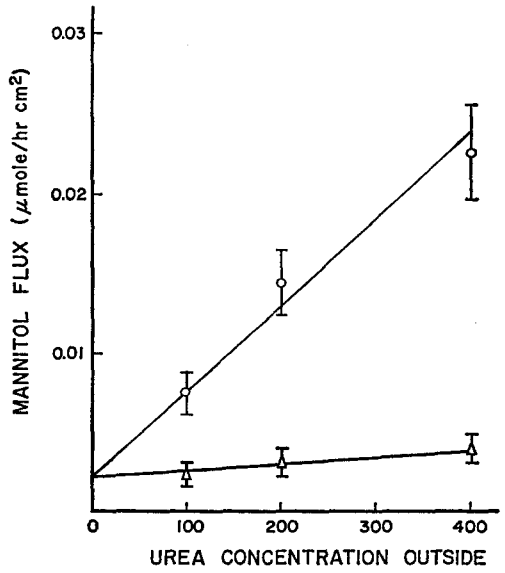

FIGURE 3. Unidirectional mannitol fluxes (○, influx; △, outflux) as a function of urea concentration of the outside solution in skins bathed in choline Ringer's. The points are average values from seven experiments and the bars represent ±1 SE.

In four experiments, the direction of the urea concentration gradient was reversed and mannitol influx and outflux were measured simultaneously. In these experiments, the outside solution was normal Na Ringer's while the inside solution was Na Ringer's plus 200 mM urea; both solutions contained 1 mM mannitol. Under these conditions, mannitol influx averaged 0.20 ± 0.03 × 10^{-3} μmole/hr cm² and outflux averaged 1.44 ± 0.24 × 10^{-3} μmole/hr cm². Thus, there was a significant net mannitol *outflux* of 1.24 ± 0.23 × 10^{-3} μmole/hr cm².

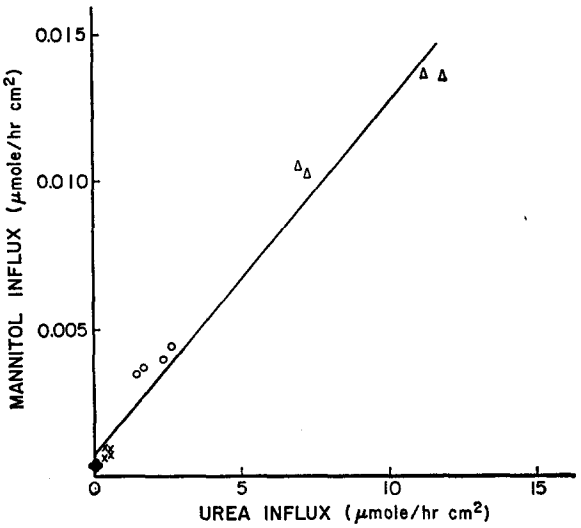

FIGURE 4. Relation between mannitol influx and urea influx. Each point represents a single period in which both fluxes were determined. Urea concentrations, (●), 1 mM; (×), 100 mM; (○), 200 mM; (△), 400 mM.

DISCUSSION

The observation of a net inward flux of mannitol when the outer surface of toad skin is exposed to solutions made hypertonic with urea is in agreement with the findings of Ussing (1) and Franz and Van Bruggen (2) in frog skin. Our results are, however, at variance with some of those reported by Ussing. We have been unable to demonstrate a relation between net mannitol flow and net Na transport in toad skin when the fluxes were measured simultaneously. In fact, urea caused an asymmetry of mannitol fluxes in skins bathed for several hours in Na-free choline Ringer's and the net flow did not differ significantly from that observed at 112 mM Na. Furthermore, the net mannitol flow was not altered significantly by ouabain even though this agent caused a substantial reduction in net Na transport. On the other hand, Ussing observed a correlation between the magnitude of net sucrose transfer and short-

circuit current in frog skin exposed to an outside solution made hypertonic with urea. In addition, he found that net sucrose flow did not occur if cyanide was added or if Na in the bathing solutions was replaced by K.

These differences could be due to species variation or to the fact that the fluxes of different solutes were measured, but these possibilities seem somewhat unlikely and other explanations should be explored. Ussing (1) noted a considerable difference between short-circuit current and net Na flux in skins bathed with hypertonic outside solutions and our data indicate a similar phenomenon in toad skin (Table II). Under these conditions, a correlation between the induced sucrose flux and current might not actually reflect a correlation between sucrose and Na fluxes. Further, examination of our results indicates that during the first 3 hr of exposure to hypertonic solution there is usually a progressive decline in net mannitol flux, due primarily to an increase in outflux. During this period, there is also a decline in net Na flux and in short-circuit current. This point is illustrated in Fig. 5 in which net mannitol fluxes are plotted against net Na fluxes observed in the first 3 hr of two experiments. Thus, if we examined only these periods, there would be a tendency for a correlation between the fluxes. If, however, net Na flux in toad skin is varied over a wide range in a single experiment by changing Na concentration, there is no clear correlation between the Na flux and mannitol flux.

In his experiments with Na-free solutions, Ussing used K to replace Na while we have used choline as the replacement ion. The explanation for the different results is not clear, but it may involve different responses of the skin to K and choline. When the inner surface of frog skin is bathed with K Ringer's, the epithelial cells swell markedly (7) and the resulting structural changes may be sufficient to alter the effect of hypertonic solutions on solute flux. Choline does not appear to penetrate the cells easily and would not be expected to cause appreciable swelling. Finally, our observations with an inhibitor of Na transport differ from those of Ussing and again there seems to be no simple explanation. However, Franz and Van Bruggen (private communication) have also observed that inhibitors do not abolish the asymmetric solute flux induced by hypertonic solutions in frog skin.

On the basis of our studies, we must conclude that the net mannitol flux observed in the presence of urea does not depend on Na transport and we have to seek alternative explanations. As demonstrated by Andersen and Ussing (8), solvent drag can give rise to a net solute flux, but such an explanation seems unlikely in the present case. The osmotic pressure difference across the skin should cause volume flow from inside to outside, the wrong direction to explain the observed asymmetry. Franz and Van Bruggen (2) have shown that the net volume flow across frog skin under these conditions is indeed in the outward direction. Although solvent drag may well influence the magnitude of the observed fluxes, it cannot account for the direction of the net man-

nitol flux in simple terms. A solvent flow could still be involved in the process if there were appropriate local currents of flow within the epithelium, but such a process is difficult to visualize particularly if Na transport is not involved. A more attractive alternative seems to be the one suggested by Franz and Van Bruggen (2) that the asymmetry is the result of a drag between solute molecules.[1] Thus, the hypertonic agent diffusing down its concentration difference is able to carry with it sufficient mannitol or other solute to account for the asymmetry. The finding (9) that a hypertonic agent such as raffinose,

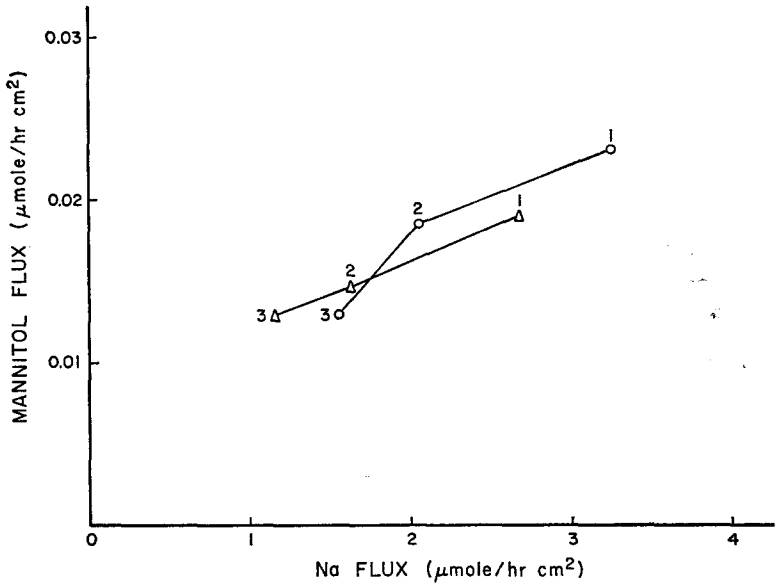

FIGURE 5. Relation between net mannitol flux and net Na flux in two experiments. Numbers adjacent to the points indicate hours after the exposure to 200 mM urea.

that does not penetrate the frog skin readily, does not cause an asymmetry of flux[2] seems to support this concept.

The data obtained in the present experiments appear to be qualitatively consistent with the hypothesis that the net mannitol flux arises, at least in part, from a coupling to the flow of urea. The existence of such coupling between solute flows has been demonstrated in free solution (11–13) and can

[1] It seems highly unlikely that the net mannitol flux can be ascribed to an effect of 200 mM urea on the activity coefficient (γ_m) of mannitol. Urea would have to *increase* γ_m by approximately six times to give rise to the observed net flux. We have been unable to find data on the effect of urea on γ_m. However, the data of Robinson and Stokes (16) indicate that 200 mM sucrose increases γ_m by only a few per cent. In a solution containing urea and sucrose, both at 0.5 M, an increase in the concentration of urea causes a decrease in the activity coefficient of sucrose (13).

[2] There are actually no direct measurements of the permeability of the skin to raffinose, but it seems reasonable to assume on the basis of molecular size that if it penetrates at all, it must do so at a rate much slower than that of urea.

be conveniently discussed within the formal framework of nonequilibrium thermodynamics (10, 11).

A simplified analysis of this type of coupling for a system involving a membrane separating solutions containing two solutes is given in the Appendix; for simplicity, effects of solvent flow on solute fluxes have been neglected. This analysis cannot be applied quantitatively to the toad skin for a number of reasons; the skin is a rather complex membrane system, more than two solutes may be involved, and solvent flow may affect the measured fluxes to a significant degree. However, certain qualitative predictions regarding solute-solute interactions are of interest with respect to the present experiments. For the case of equal mannitol concentrations in the two solutions, net mannitol flux, J_m, would be given by

$$J_m = (RTw_{12})\Delta c_u \qquad (1)$$

in which w_{12} is a permeability coefficient expressing an interaction between mannitol and urea, and Δc_u is urea concentration difference. The data shown in Fig. 3 are in agreement with this type of expression since they indicate that net mannitol flux is proportional to Δc_u.

The observation that a net outflux of mannitol occurs when the inside solution is made hypertonic with urea is also qualitatively consistent with the behavior expected on the basis of equation 1; reversal of the direction of Δc_u should lead to reversal of the direction of J_m. It is clear, however, that this description is not adequate in a quantitative sense because equation 1 predicts that J_m should have the same magnitude in either direction for the same Δc_u, while the data indicate that when $\Delta c_u = 200$ mM net mannitol influx is considerably greater than the outflux observed when $\Delta c_u = -200$ mM. This polarity in the effect of urea may be due in part to the complex nature of the skin and its different response to hypertonic solutions at the inside and outside. In the presence of 200 mM urea inside, the potential difference and short-circuit current remain high whereas these parameters decrease markedly with 200 mM urea outside. In addition, the unidirectional mannitol fluxes observed with hypertonic outside solution are considerably greater than those found with hypertonic inside solution.

This type of solute-solute interaction could also lead to a relation such as that shown in Fig. 4 between mannitol influx and urea flux. For the simplified system discussed in the Appendix, the predicted relation is

$$J_m^i = (RTw_{11})\, c_m + \left(\frac{w_{12}}{w_{22}}\right) J_u \qquad (2)$$

in which J_m^i is unidirectional mannitol influx, c_m is mannitol concentration, and w_{11} and w_{22} are the "straight" permeability coefficients for mannitol and

urea, respectively. According to equation 2, mannitol influx should increase with increasing net urea flux as observed.[3] If we assume that equation 2 describes, to a first approximation, events in toad skin, the slope of the line in Fig. 4 should give an estimate of the extent of interaction between mannitol and urea expressed by the ratio w_{12}/w_{22}. The value obtained is 1.2×10^{-3}. This degree of interaction is smaller than some of the values observed in free solution, in which the ratio equivalent to w_{12}/w_{22} is a ratio of generalized diffusion coefficients (11) D_{12}/D_{22}. Ellerton and Dunlop (12, 13) have recently reported values of these coefficients for sucrose and mannitol and for sucrose and urea. At solute concentrations of 0.25 M, the values of D_{12}/D_{22} obtained varied from 0.146 to 0.0022 depending on which solutes are designated 1 and 2. Thus, it appears that the observed mannitol flux could be explained in terms of solute-solute interaction without the necessity of postulating particularly exaggerated cross-effects in the skin. In addition, this estimate of w_{12}/w_{22} may give a maximum value. The over-all permeability of the skin appears to increase as urea concentration is increased; in the experiments shown in Fig. 4, urea permeability increased from 0.0019 cm/hr at 1 mM to 0.023 cm/hr at 400 mM urea (see also Na outflux in Table II). Under these conditions, it seems unlikely that mannitol permeability, represented in equation 2 by w_{11}, would remain constant. Any correction for increase in J_m^i because of an increased permeability will tend to reduce w_{12}/w_{22} from the value given.

Again it is clear that this simplified treatment does not provide an adequate quantitative description for the skin. The appropriate form of equation 2 would predict that mannitol outflux should decrease with increasing urea flux but this effect was not observed (Fig. 3). However, the effect could be obscured by a general increase in permeability. That is, in the absence of coupling between urea and mannitol, outflux would have increased markedly due to a permeability change, but the coupling serves to reduce the magnitude of the increase. For the observed values of J_u, a ratio of w_{12}/w_{22} of the order of 1×10^{-3} would be sufficient to obscure the effect on outflux of a 10-fold increase in mannitol permeability.

Although these results cannot be considered entirely definitive they seem consistent with the concept of a drag effect between diffusing solutes. We have been unable to demonstrate any clear relation between Na transport and the net mannitol flux induced by urea but our results can be explained qualitatively by postulating a relatively small cross-coefficient relating mannitol flow to urea flow. Thus, the net mannitol flux may arise as a result of frictional interaction with urea that is diffusing across the skin down a large

[3] The data in Fig. 4 are actually for urea influx. However, since the urea concentration in the inside solution was 1 mM, the influx observed when the outside urea concentration was 100 mM or greater is approximately equal to the net flux.

concentration difference. However, further work is necessary to test this hypothesis. Precise data on the effects of solvent drag on the fluxes are required as is information on the permeability changes caused by hypertonic solutions. In addition, effects of other solutes should be investigated in more detail and the influence of changes in concentration of the "dragged" solute should be examined.

APPENDIX

In order to obtain some insight into the behavior of solute-solute interactions, we consider a simple membrane bathed on both sides by solutions containing only urea and mannitol as solutes. An extension of the approach used by Kedem and Katchalsky (17) (see for example, equations 17 of reference 18) leads to the following expressions for the net flows of mannitol, J_m, and urea, J_u :

$$J_m = \bar{c}_m(1 - \sigma_m)J_v + RTw_{11}\Delta c_m + RTw_{12}\Delta c_u \qquad (A1)$$

$$J_u = \bar{c}_u(1 - \sigma_u)J_v + RTw_{21}\Delta c_m + RTw_{22}\Delta c_u \qquad (A2)$$

in which J_v is volume flow, $\bar{c}$ is mean concentration (17), σ is the reflection coefficient (17), and Δc indicates $c^{out} - c^{in}$. The subscripts u and m denote urea and mannitol and the w_{ij} are generalized permeability coefficients. In order to illustrate possible effects of solute-solute interactions, we shall ignore the influence of volume flow on the solute fluxes by assuming that $J_v = 0$. While these effects could clearly modify the magnitude of the solute fluxes, the qualitative conclusions of interest for the present case are not seriously altered. Under conditions in which $\Delta c_m = 0$, equation A1 reduces to

$$J_m = (RTw_{12})\Delta c_u \qquad (A3)$$

indicating that the net mannitol flux would be proportional to the urea concentration difference.

This approach also provides a relation between mannitol influx and urea flux. The details of this description of tracer fluxes have been discussed previously (14, 15) so that only a summary is given. We are interested in the flux of labeled mannitol, J_m^* which, in the absence of solvent drag effects, is given by

$$J_m^* = RTw_{11}^*\Delta c_m^* + RTw_{12}^*\Delta c_u \qquad (A4)$$

in which the superscript * denotes the labeled species. Multiplying both sides of equation A 4 by c_m/c_m^* where c_m is the concentration of unlabeled mannitol and c_m^* is the concentration of tracer on the hot side, we obtain

$$\frac{J_m^* c_m}{c_m^*} = RTw_{11}^* \frac{c_m}{c_m^*}\Delta c_m^* + RTw_{12}^* \frac{c_m}{c_m^*}\Delta c_u \qquad (A5)$$

Under the conditions used in our experiments, $\Delta c_m^* \cong c_m^*$ and the quantity $J_m^* c_m/c_m^*$

is the unidirectional mannitol flux, J_m^i, calculated by conventional methods. Further, the assumption that tracer and bulk mannitol are indistinguishable requires (15) that $w_{12}^*/c_m^* = w_{12}/c_m$ so that equation A 5 becomes

$$J_m^i = RTw_{11}^* c_m + RTw_{12}\Delta c_u \qquad (A6)$$

Since urea flux, J_u, is given by $J_u = RTw_{22}\Delta c_u$ equation A 6 becomes

$$J_m^i = RTw_{11}^* c_m + \left(\frac{w_{12}}{w_{22}}\right) J_u \qquad (A7)$$

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